

Mathematical Model of Steroidogenesis in Fathead Minnow Ovaries to Predict Biochemical Response to Endocrine Active Compounds

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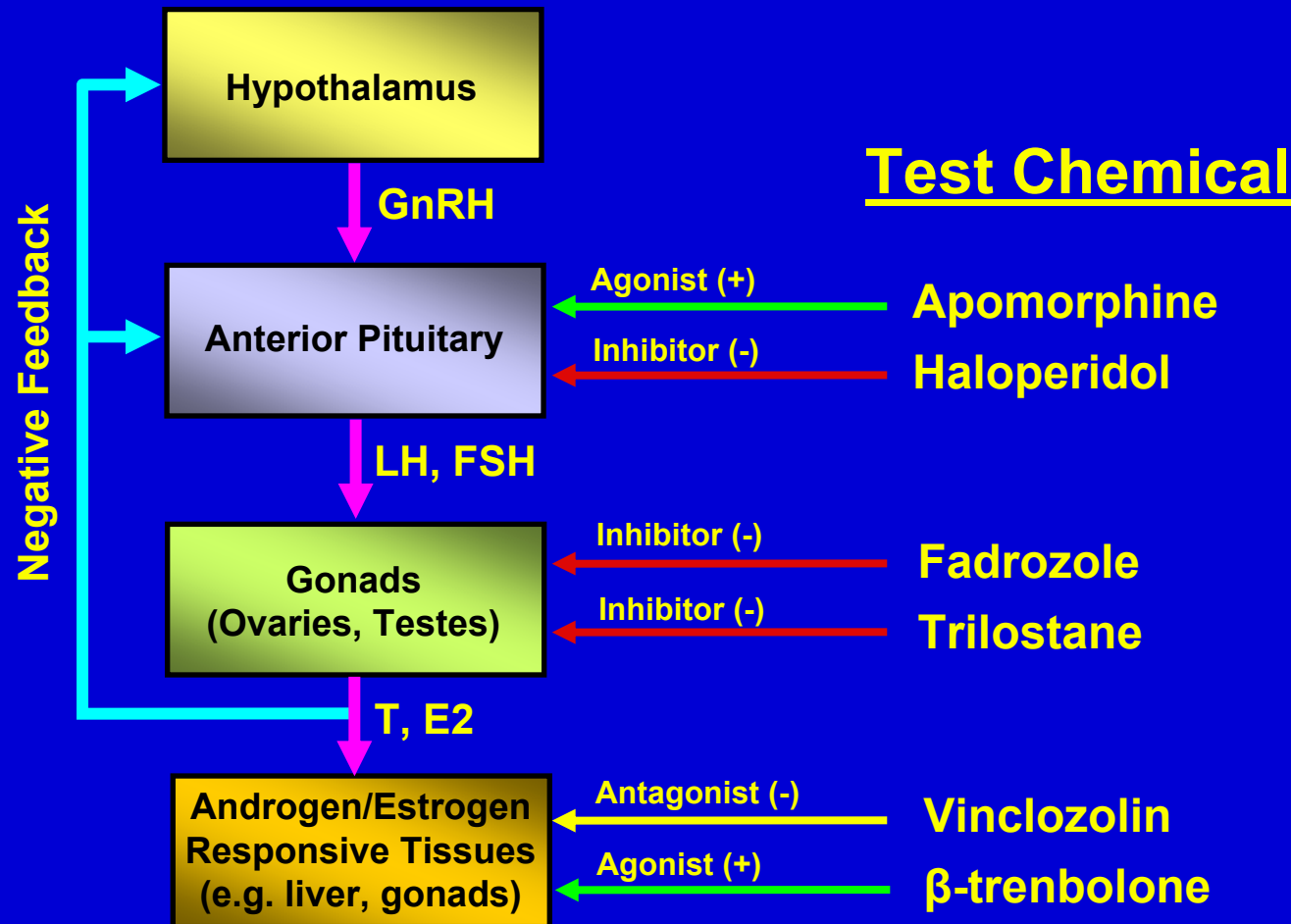


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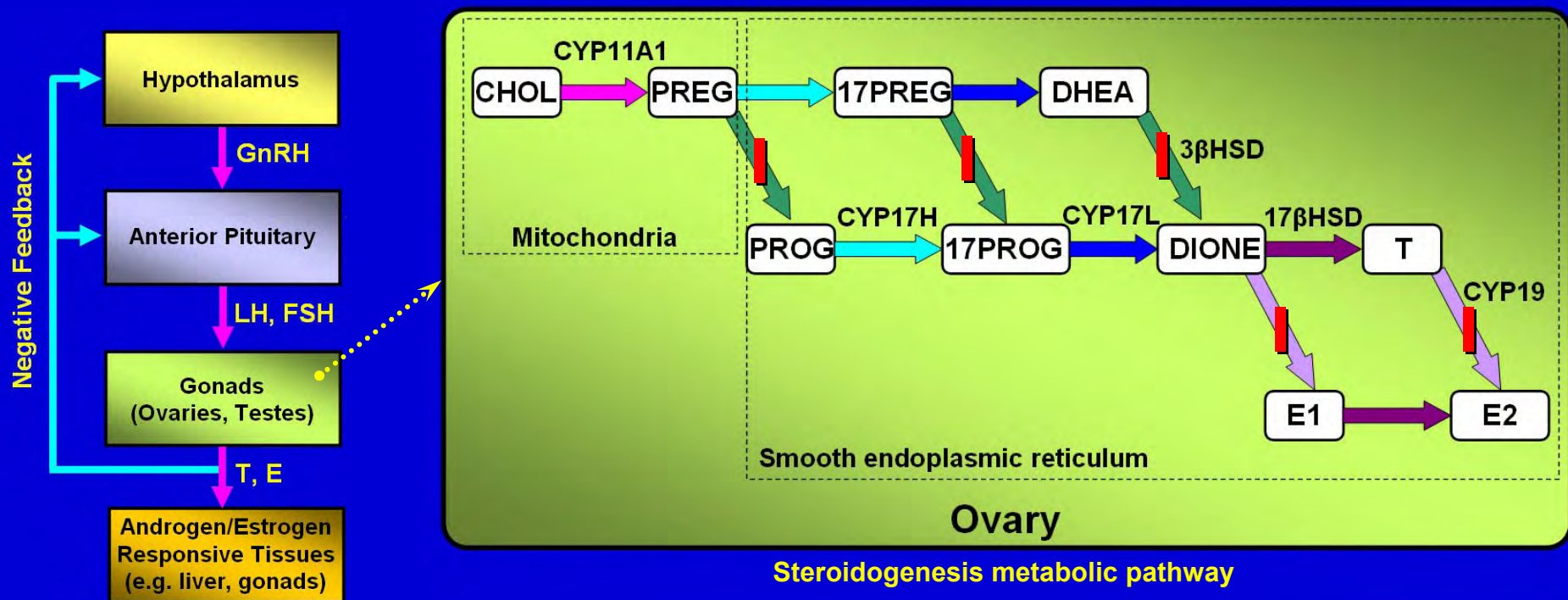
Outline

- **Effects of EAC on steroidogenesis**
- **Computational model of ovarian steroidogenesis to predict biochemical response for baseline and fadrozole studies**
 - *In vitro* steroidogenesis assay with ovary explants
 - Ovarian steroidogenesis model with enzyme inhibition by fadrozole
 - Steady-state analysis
 - Estimation of parameters
 - Assessment of model fit
 - Sensitivity analysis
- **Summary**

Effects of EAC on HPG Axis



Effects on Steroid Metabolism



Chemical	Mode of Action	Source
Fadrozole	Inhibit CYP19	Breast cancer therapy
Trilostane	Inhibit 3βHSD	Cushing's disease treatment

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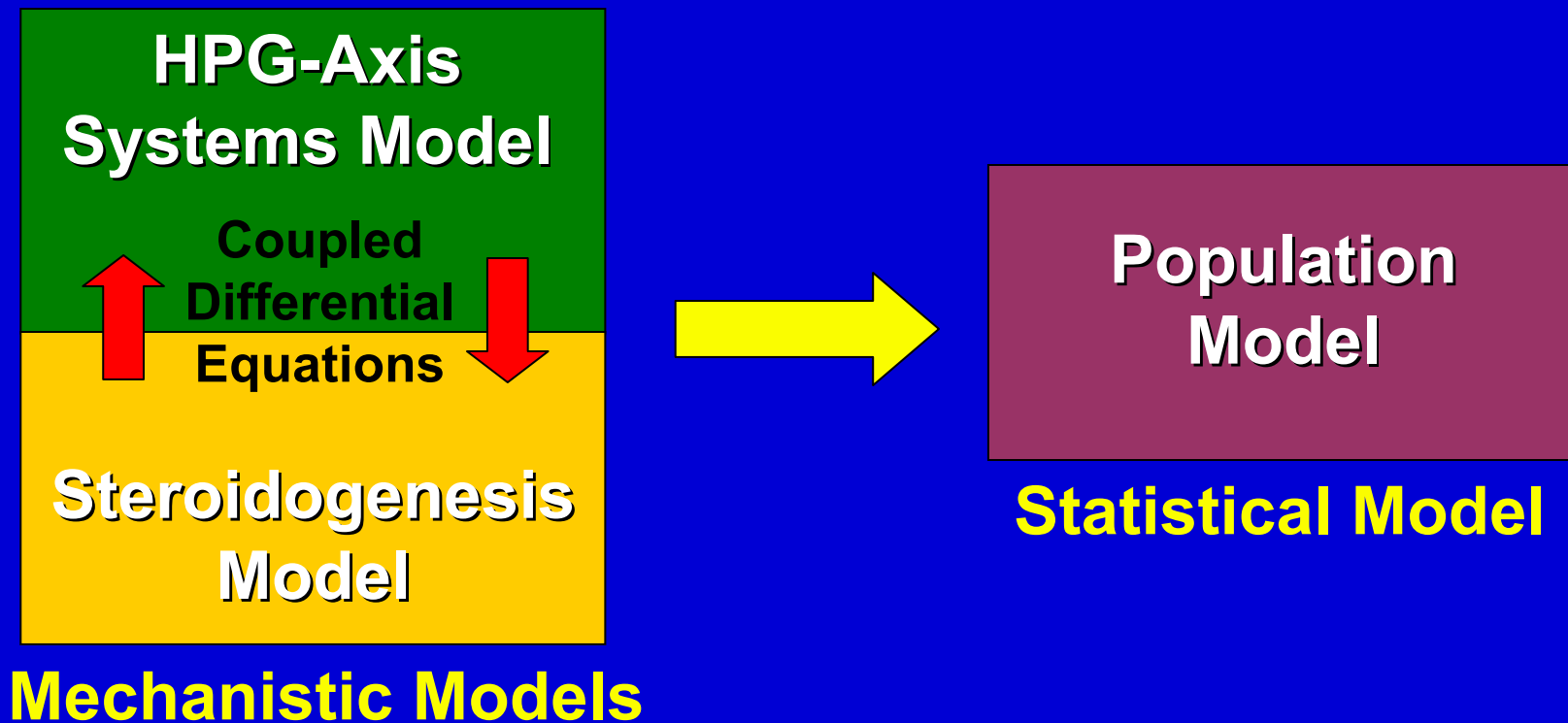
Mechanistic Computational Steroidogenesis Model



- Improve understanding of dose-response behavior for EAC
- Help define mechanism of actions for poorly characterized chemicals
- Serve as a basis to identify predictive biomarkers (patterns of steroid changes) indicative of exposure and adverse effects
- Support environmental human health and ecological risk assessments
- Help screen drug candidates based on steroid effect in early phase of drug development

Population Effects Model

Coupled Systems Model



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Endocrine Disruption in Fish



Fathead minnow

- Convincing evidence that fish are affected at individual and population levels
- Fish may serve as effective environmental sentinels for possible effects in other vertebrates
- Evolutionarily conserved HPG axis

Objective

Create a computational model of ovarian steroidogenesis and estimate parameters to predict synthesis and secretion of T and E2 for *in vitro* baseline and fadrozole studies

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In Vitro Steroidogenesis Experiments: Baseline



Small fish culture facility

- Dissect fish ovary
- Incubate ovary in medium supplemented with cholesterol
- Collect medium at six time points over 31.5 hr
- Measure medium concentrations of testosterone (T) and estradiol (E2) using radioimmunoassay



Fathead minnows

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In Vitro Steroidogenesis Experiments: Fadrozole



Small fish culture facility

- Dissect fish ovary
- Incubate ovary in medium supplemented with cholesterol and five fadrozole (FAD) concentrations
- Collect medium at 14.5 hr
- Measure medium concentrations of testosterone (T) and estradiol (E2) using radioimmunoassay



Fathead minnows

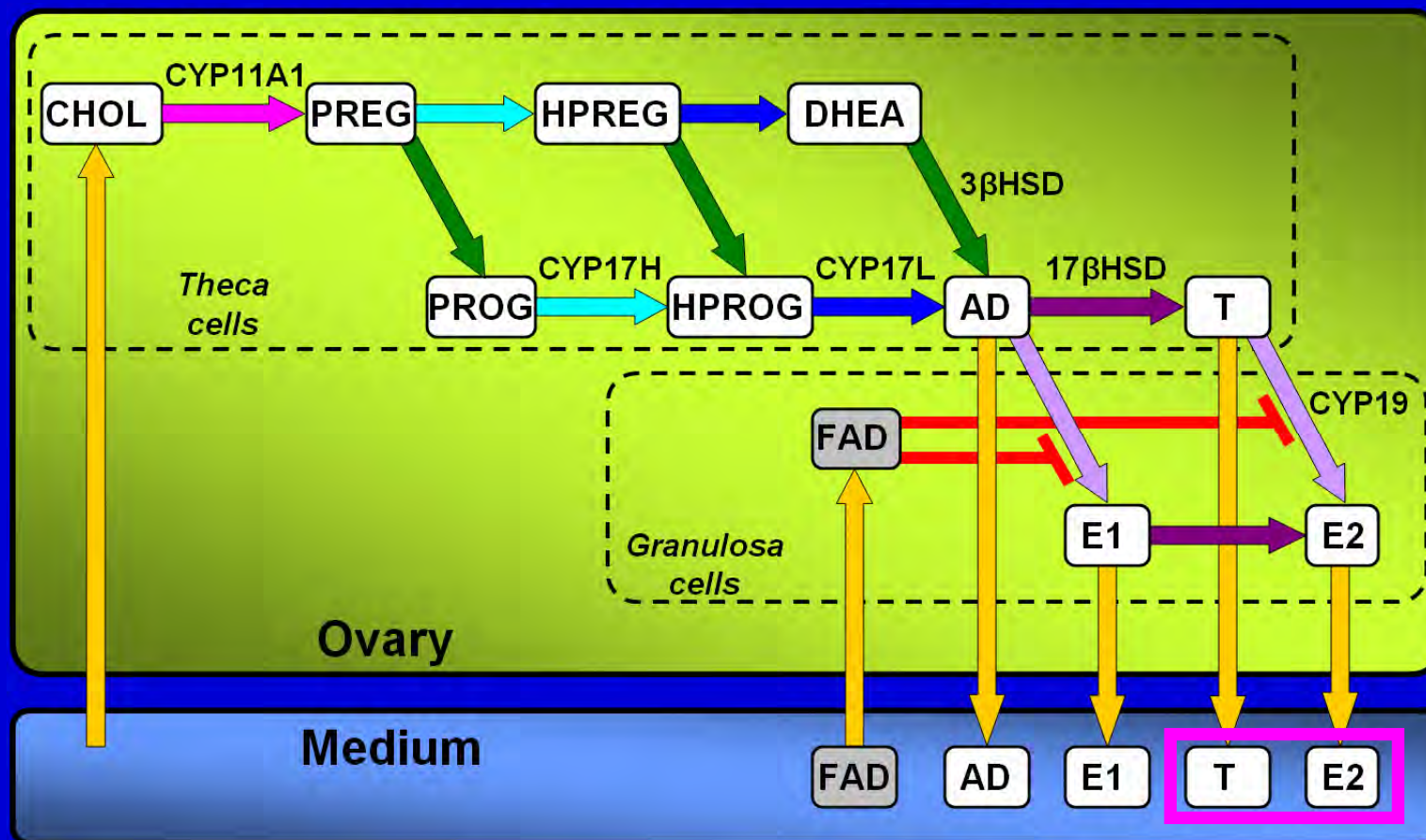
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Conceptual Steroidogenesis Model



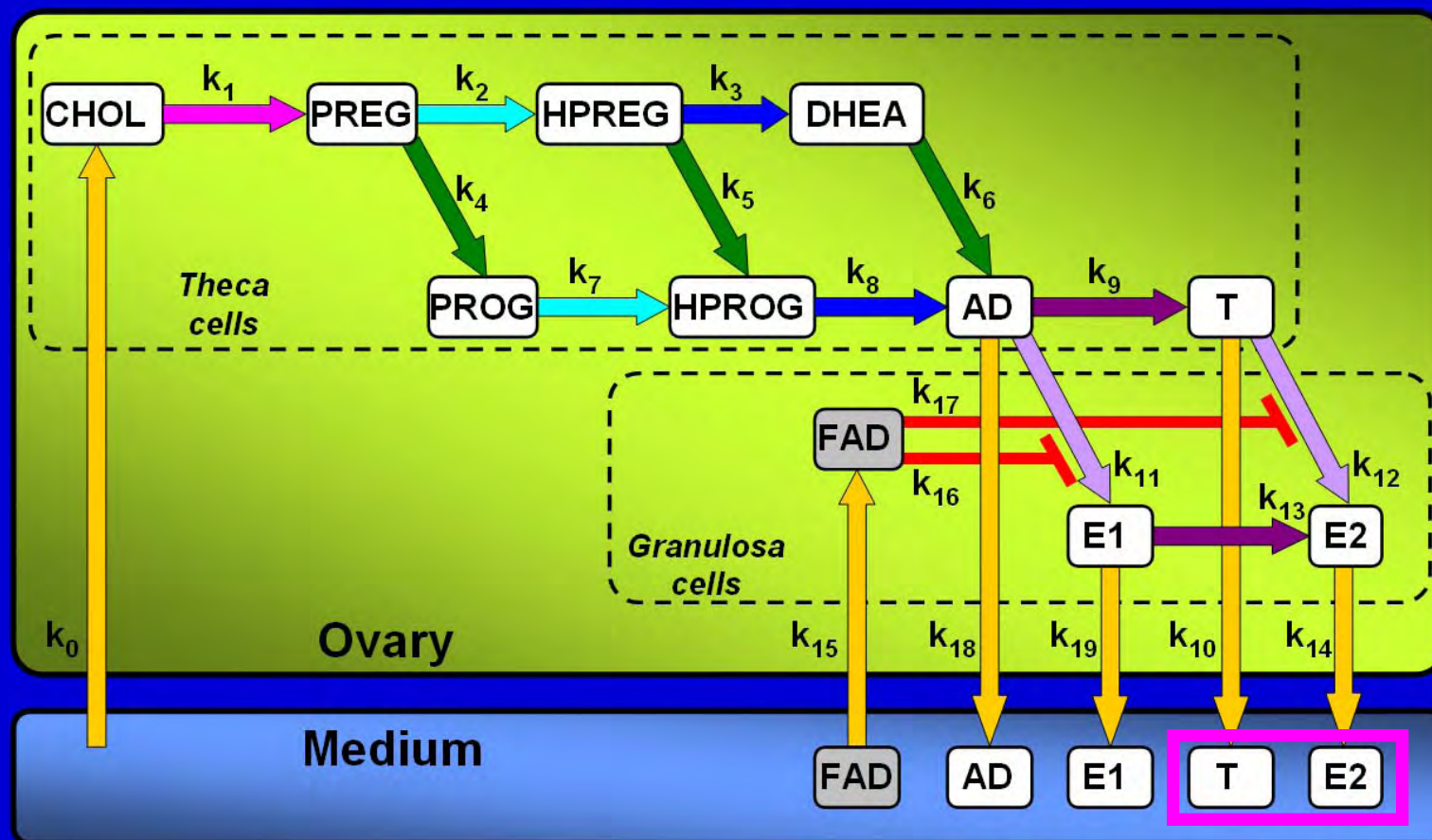
- 6 unique enzymes
- 12 enzymatic reactions
- 4 secreted steroids

Measured

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Computational Steroidogenesis Model



- 6 transport rates
- 12 first-order enzymatic reaction rates
- 2 enzyme inhibition constants

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Dynamic Mass Balances

Ovary:
$$V_{\text{ovy}} \frac{dC_{x,\text{ovy}}}{dt} = \underbrace{P_{x,\text{ovy}} - U_{x,\text{ovy}}}_{\text{Net metabolic rate}} + \underbrace{I_{x,\text{ovy}} - S_{x,\text{ovy}}}_{\text{Net uptake rate}}$$

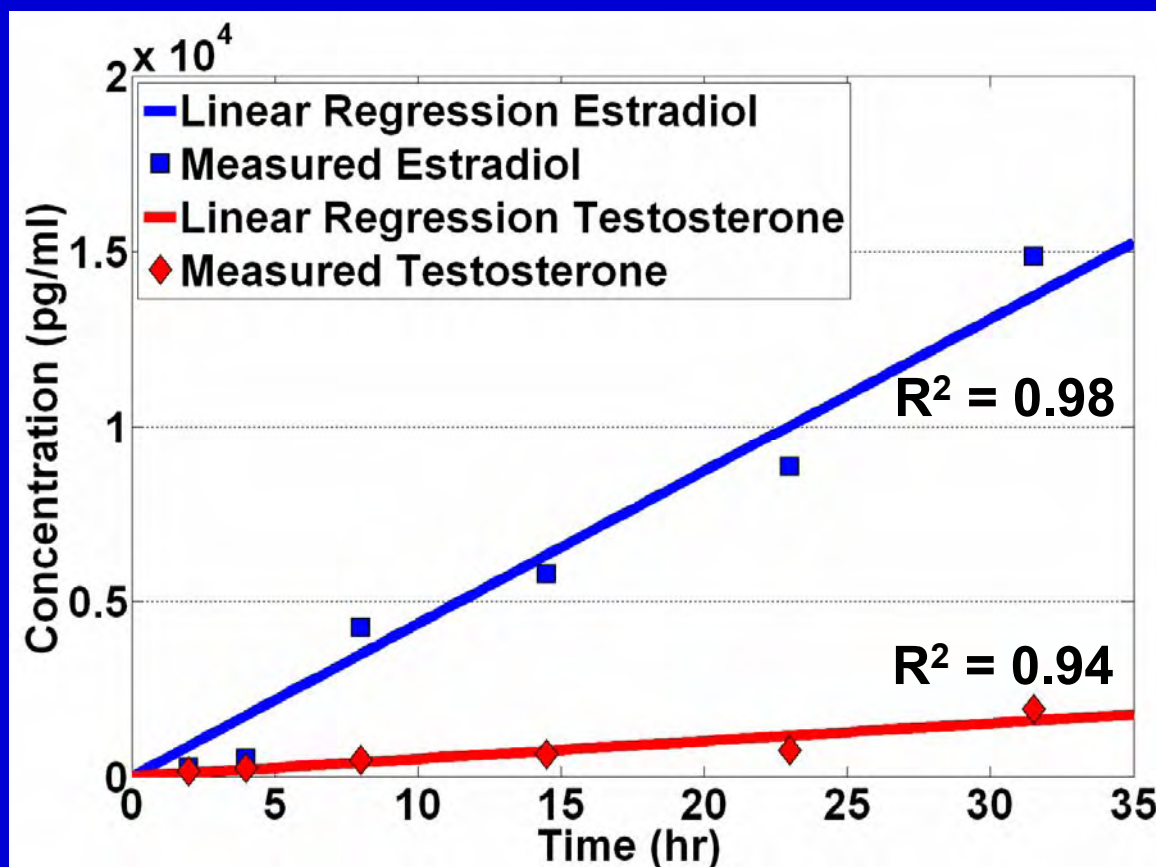
Medium:
$$V_{\text{med}} \frac{dC_{x,\text{med}}}{dt} = \underbrace{S_{x,\text{ovy}}}_{\text{Ovary secretion rate}}$$

- Yields a system of coupled differential equations
- 20 model parameters

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Measured Steroids from Baseline Study



- Good evidence steroid synthesis is operating near steady-state during experiments
- Steady-state assumption reduces model complexity

Steady-State Analysis

- Set differential equations in ovary to zero to yield algebraic equations
- Determined analytical solutions for testosterone ($C_{T,med}$) and estradiol ($C_{E2,med}$) in medium
- Solutions depend on 11 out of 20 parameters

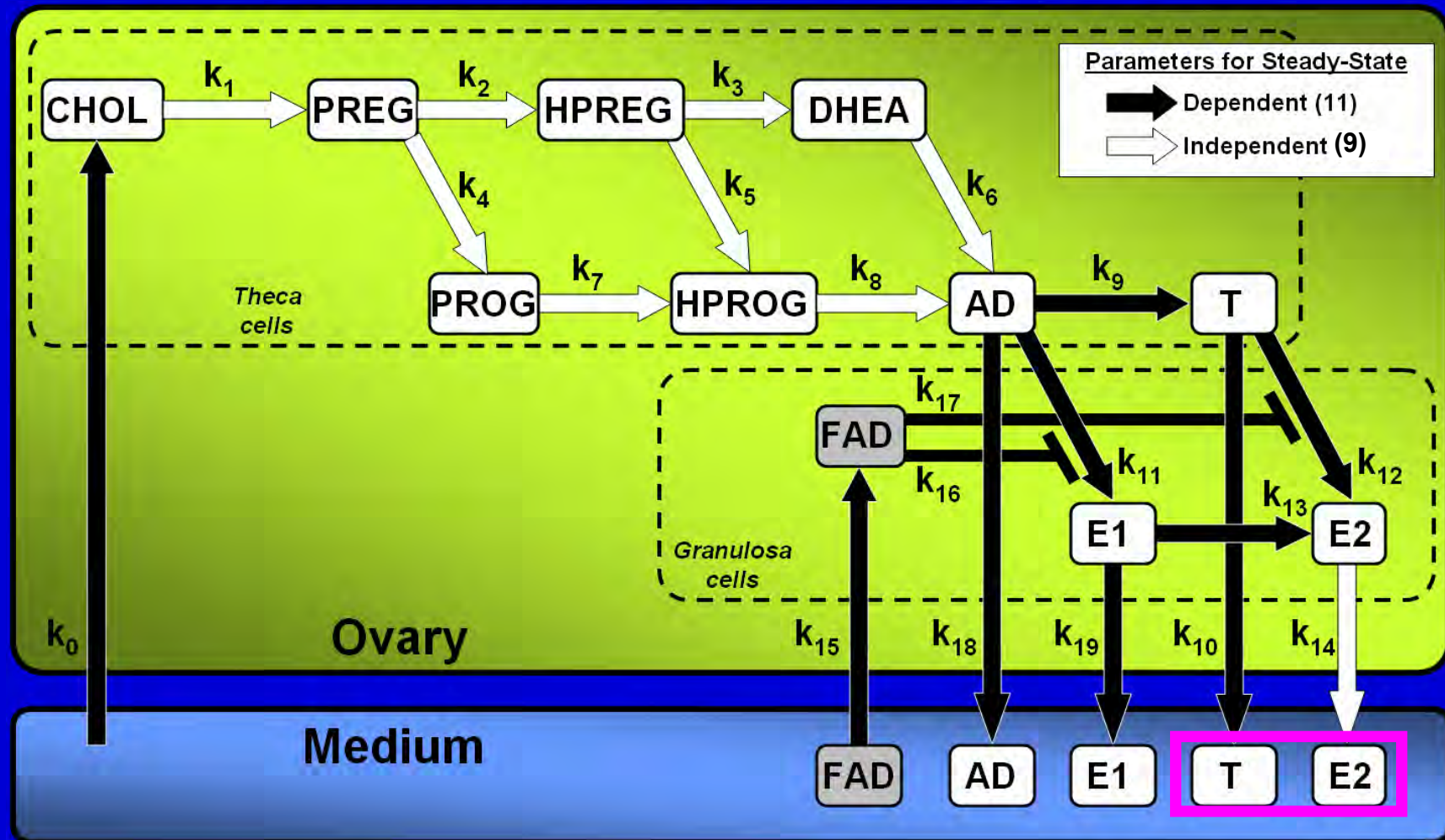
$$C_{T,med}(t) = \frac{k_0 k_9 k_{10} (k_{16} + k_{15} C_{FAD,med}) (k_{17} + k_{15} C_{FAD,med}) t}{D_1 D_2}$$

where: $D_1 = k_9 k_{16} + k_9 k_{15} C_{FAD,med} + k_{11} k_{16} + k_{18} k_{16} + k_{18} k_{15} C_{FAD,med}$

$$D_2 = k_{10} k_{17} + k_{10} k_{15} C_{FAD,med} + k_{12} k_{17}$$

$$C_{FAD,med} = \text{fadrozole conc. in medium}$$

Steady-State Analysis



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Parameter Estimation

Cost function:

$$J(\bar{k}) = \sum_{d=1}^6 \sum_{i=1}^{n_d} \left(\underbrace{C_{T,med}^{d,i}}_{\text{Measured}} - \underbrace{C_{T,med}(t_i; C_{FAD,med}^d, \bar{k})}_{\text{Model-Predicted}} \right)^2 + \left(\underbrace{C_{E2,med}^{d,i}}_{\text{Measured}} - \underbrace{C_{E2,med}(t_i; C_{FAD,med}^d, \bar{k})}_{\text{Model-Predicted}} \right)^2$$

where:

- $C_{T,med}^{d,i}$ = measured testosterone for d^{th} FAD dose at i^{th} time
- $C_{T,med}$ = model-predicted testosterone
- $C_{E2,med}^{d,i}$ = measured estradiol for d^{th} FAD dose at i^{th} time
- $C_{E2,med}$ = model-predicted estradiol
- $C_{FAD,med}^d$ = measured fadrozole for d^{th} FAD dose

- Applied a nonlinear iterative optimization algorithm
- Simultaneously estimated parameters using data from baseline and fadrozole-exposure studies

Estimated Parameters

Ovary Uptake of Cholesterol and Fadrozole

k_0	15401.470	pg ml ⁻¹ hr ⁻¹
k_{15}	0.0015	Partition coefficient (dimensionless)

Secretion of Testosterone and Estradiol

k_{10}	1726.553	hr ⁻¹
k_{18}	149.301	hr ⁻¹
k_{19}	102.171	hr ⁻¹

First-order Enzyme Kinetics with Inhibition by Fadrozole

k_9	0.509	hr ⁻¹	* Literature values from fish experiments
k_{11}	5.8*	hr ⁻¹	
k_{12}	3.2*	hr ⁻¹	
k_{13}	356.217	hr ⁻¹	FAD inhibition constants
k_{16}	8143.017	pg ml ⁻¹	
k_{17}	4671.198	pg ml ⁻¹	

Breen MS *et al.* Annals of Biomedical Engineering, 2007

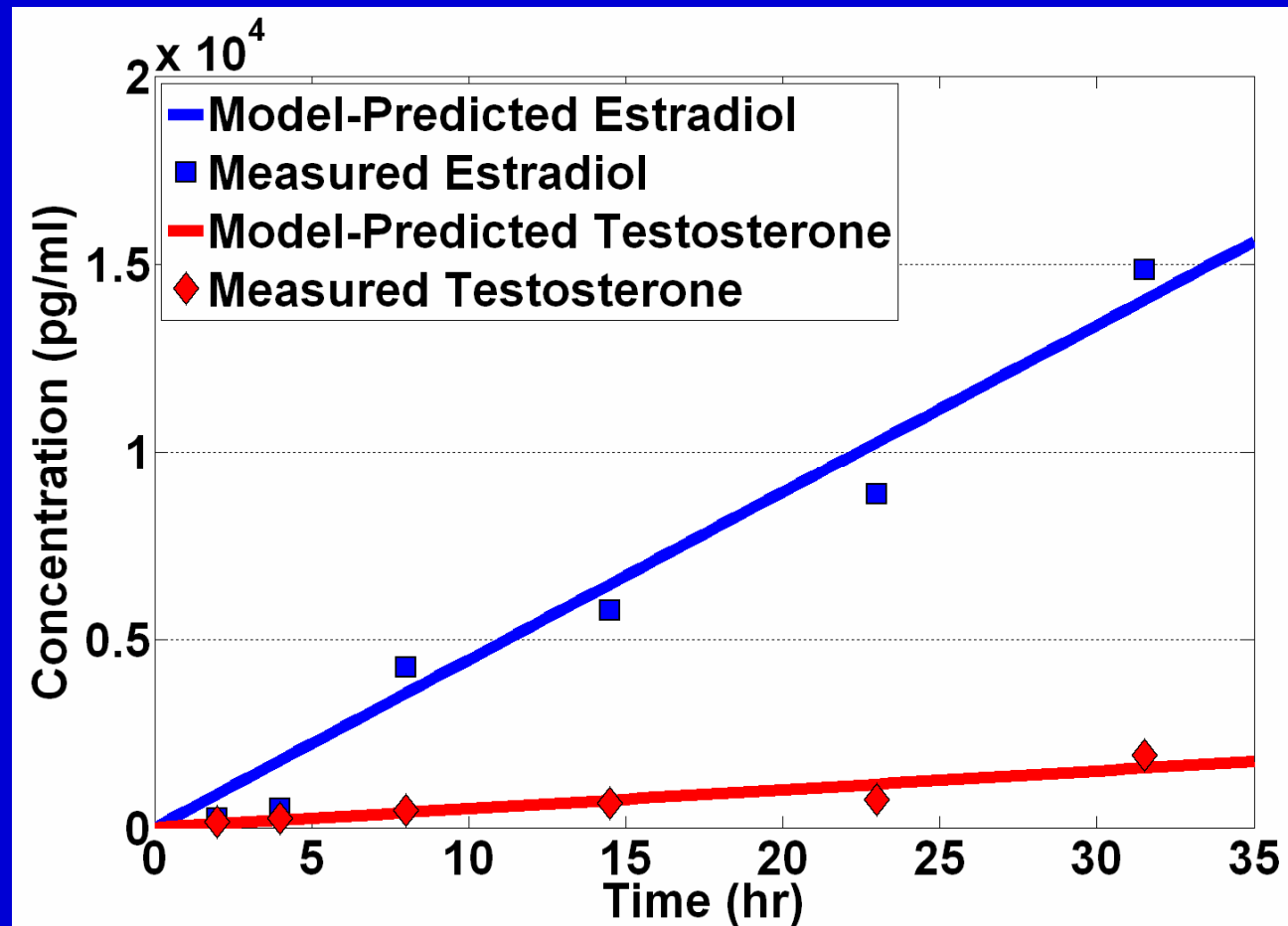
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Evaluation of Model Fit: Baseline Study

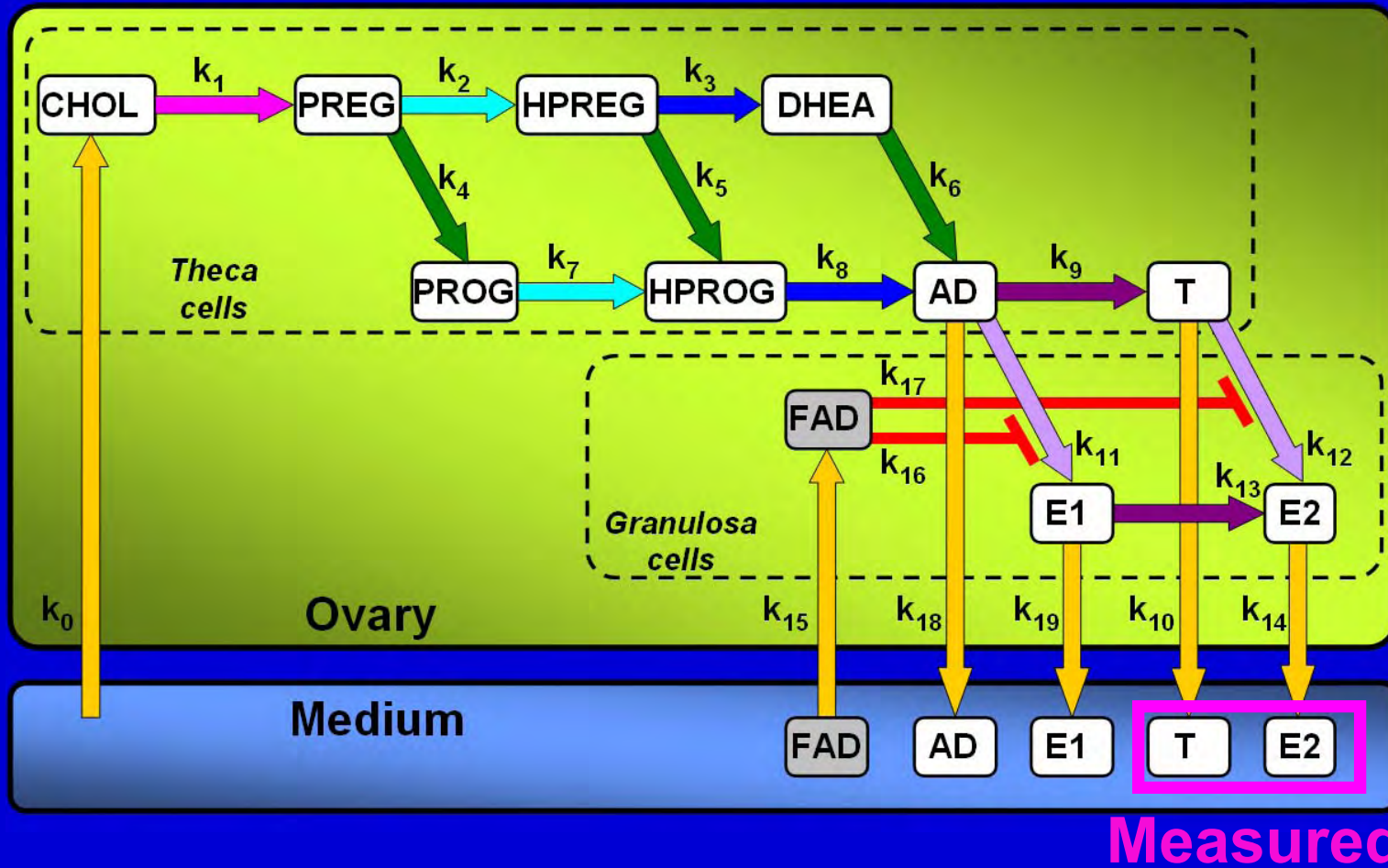


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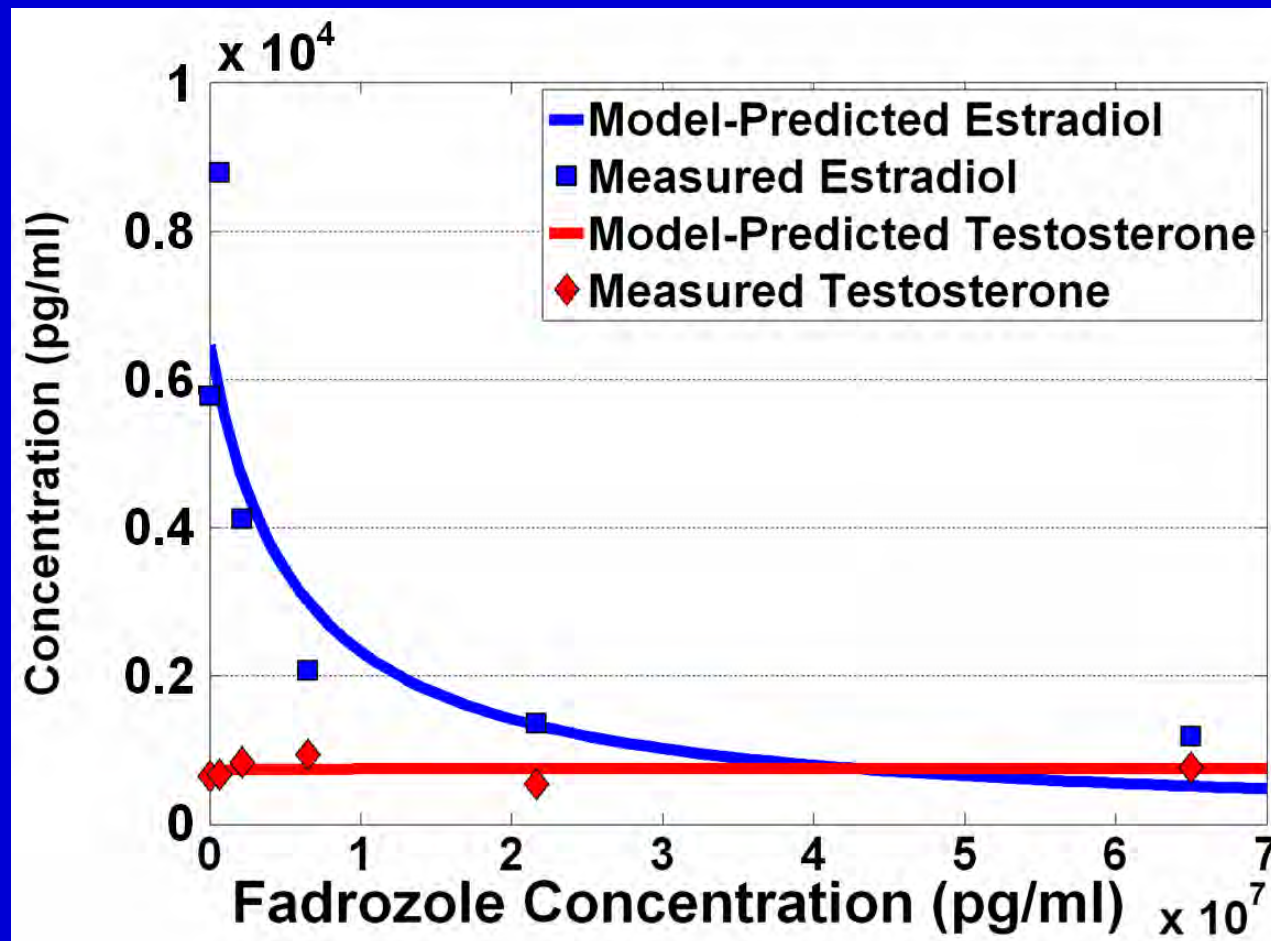
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Fadrozole Study



- Expect E2 to decrease with increasing FAD
- Expect T to increase with increasing FAD

Evaluation of Model Fit: Fadrozole Study

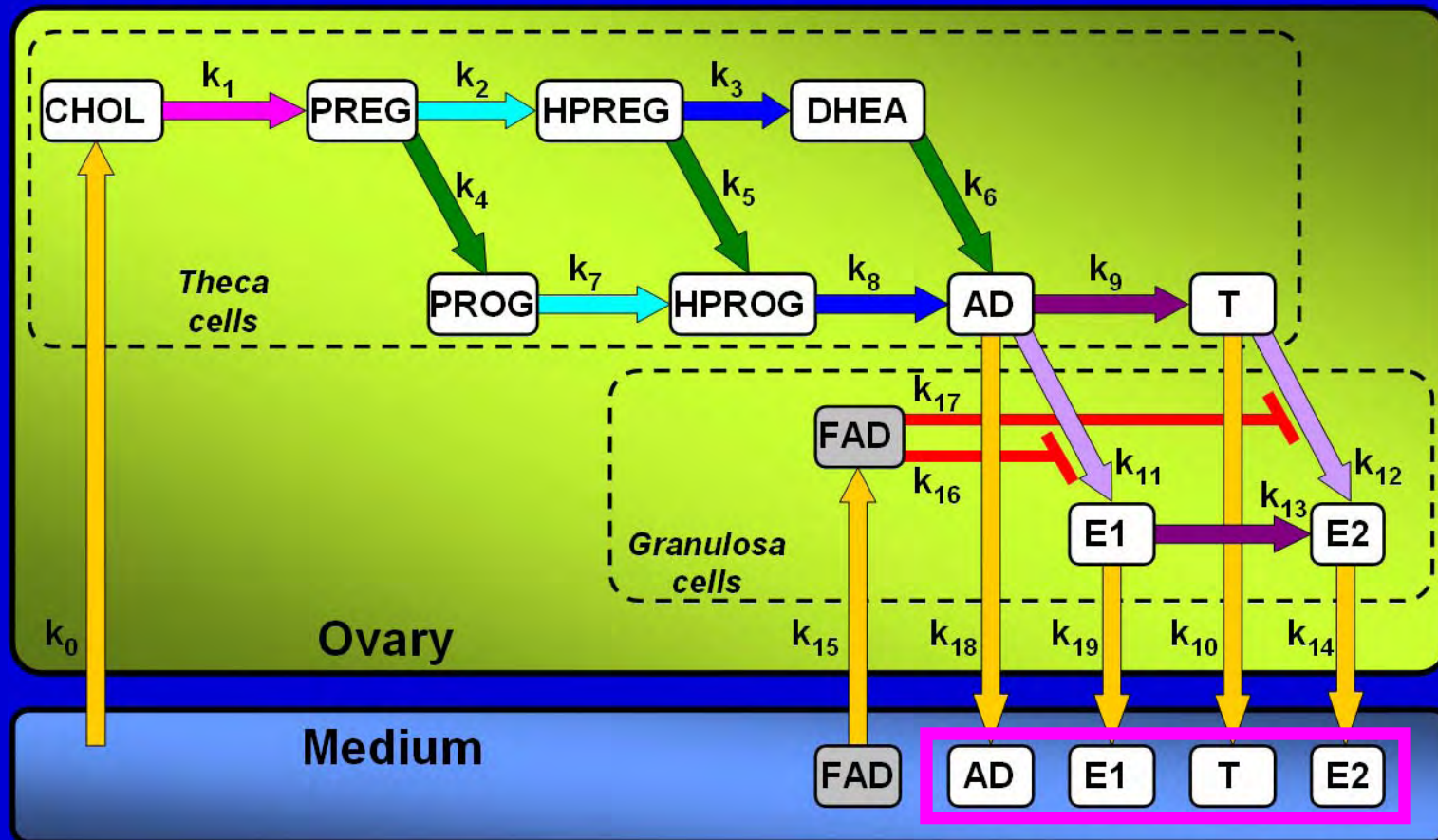


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Model-based Experimental Design



Model-based Hypothesis:

T does not change with increasing FAD due to large secretion of AD into medium

Measurements in next experiment

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Sensitivity Analysis

**Relative
Sensitivities:**

$$RST_{k_i} \left(C_{T,med}, k_i \right) = \frac{\delta C_{T,med}}{\delta k_i} \left(\frac{k_i}{C_{T,med}} \right)$$

$$RSE2_{k_i} \left(C_{E2,med}, k_i \right) = \frac{\delta C_{E2,med}}{\delta k_i} \left(\frac{k_i}{C_{E2,med}} \right)$$

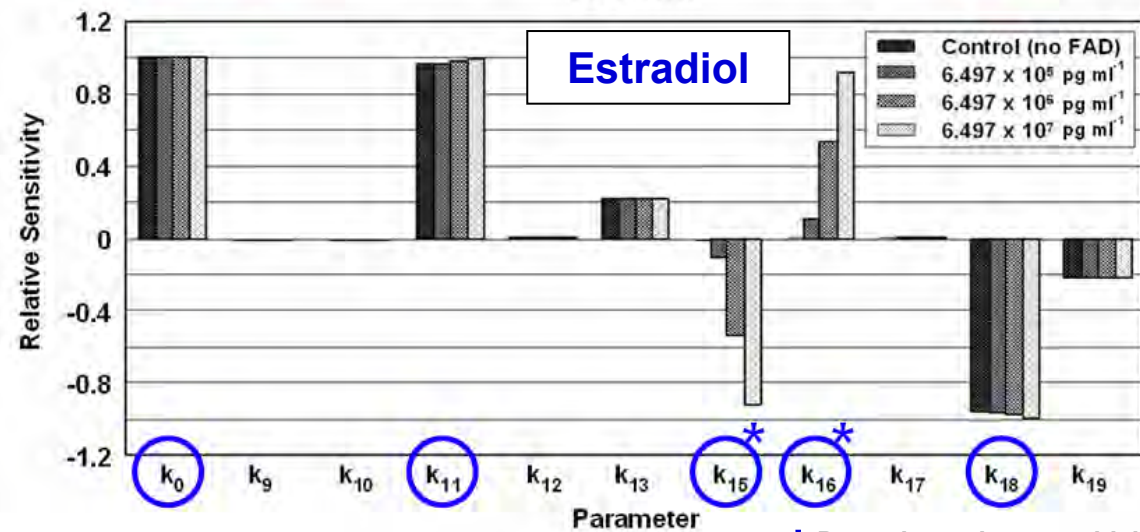
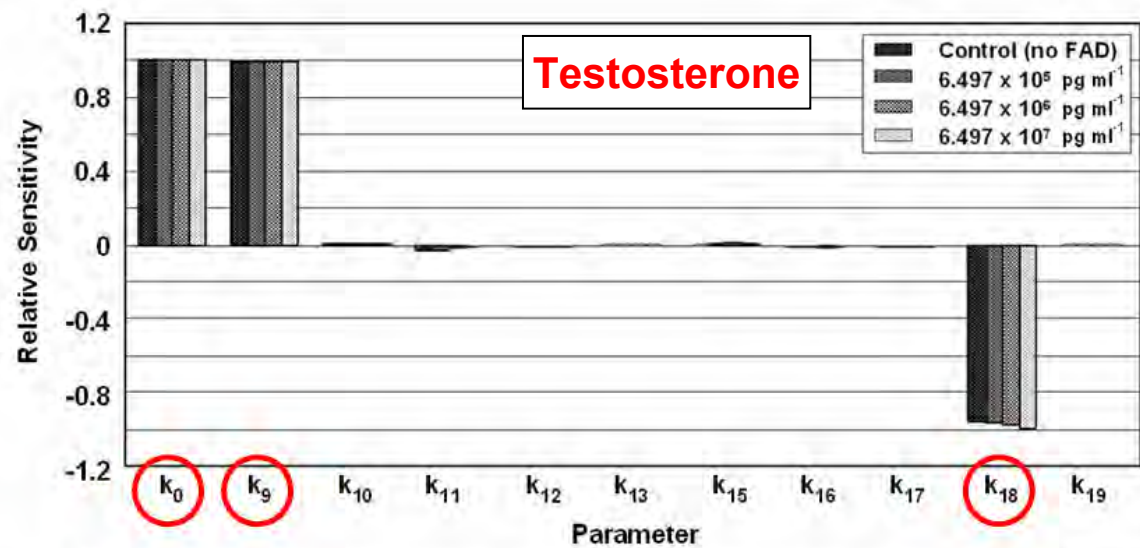
where: $C_{T,med}$ = model-predicted testosterone

$C_{E2,med}$ = model-predicted estradiol

k_i = i^{th} parameter

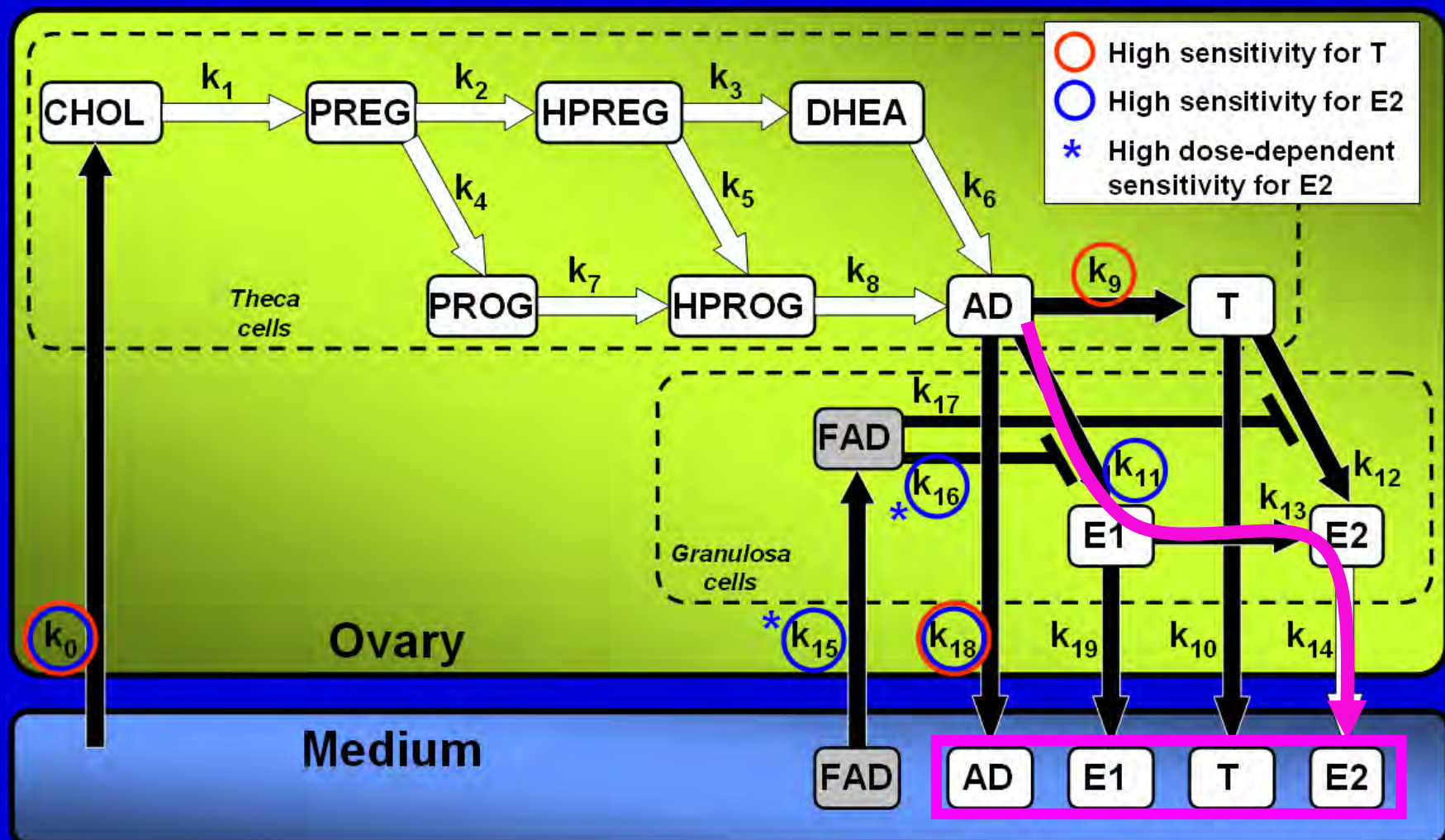
- Analytically determined partial derivatives with respect to each parameter
- Evaluated relative sensitivities for control and each fadrozole dose

Sensitivity Analysis



* Dose-dependent sensitivity

Sensitivity Analysis



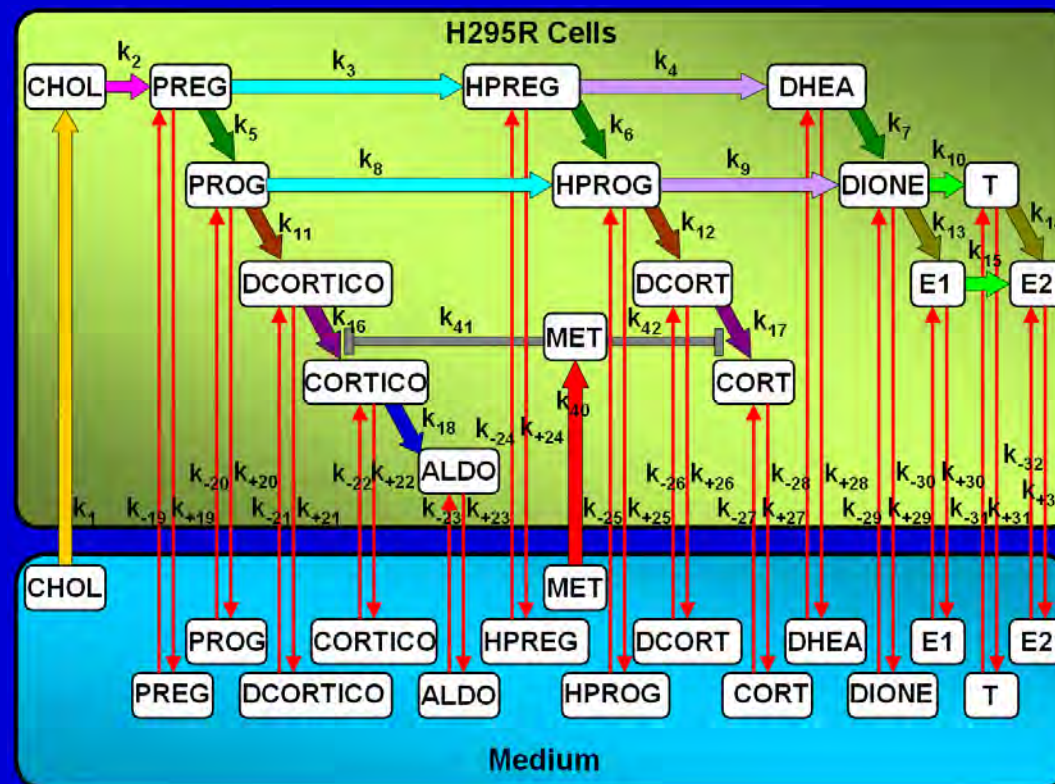
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Summary

- Steroidogenesis model can predict T and E2 concentrations, *in vitro*, while reducing model complexity with steady-state assumption
- Sensitivity analysis indicates E1 pathway as preferred pathway for E2 synthesis
- Model and sensitivity analysis support hypothesis that T is unchanged with increasing FAD due to large secretion of AD into medium
- Mechanistic model can help plan experiments and better understand dose-response behavior of chemicals that alter activity of steroidogenic enzymes
- This capability could help define mechanisms of action for poorly characterized chemicals in support of environmental risk assessments

- **Poster #3: Mechanistic Computational Model of Steroidogenesis in H295R Cells: Predicting Biochemical Response to Endocrine Active Chemicals**
- **Session IV: Plenary Session: Mining Minnows and Building Models: An Integrated Systems Biology Approach to Link Mechanism of Action to Ecologically-Relevant Outcomes, Daniel Villeneuve**



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